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Synthetic Pores as Sensors: Focus on Zinc Filters for IP_7 and Phytate Contents in Lentils, Almonds, and Soybeans

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Abstract: Recent experimental evidence confirms synthetic pores as operational multicomponent sensors in complex matrices. This work in practice is summarized, with emphasis on topics of current concern which exemplify the use of enzymes as signal generators in differential sensing applications, and the importance of carefully conceived additives such as reactive signal amplifiers or filters.

Keywords: Fluorometric sensing · Inositol phosphates · Phytase · Synthetic pores

The grand vision of pores as sensors has failed to deliver in practice for decades because of the persistent incompatibility with multicomponent sensing in complex matrices.[1] Either the sensors worked for just one or two analytes, or the background noise obscured any distinct signal. Last year, we have solved this problem with the creation of reactive signal amplifiers; molecules conceived to capture otherwise elusive analytes after enzymatic signal generation and drag them into the pore for signal transduction.[2] This approach has provided access to synthetic pores that can sense sweet, sour, and umami flavors in samples from the supermarket. Artificial tongues were an obvious topic to elaborate on multicomponent sensing in complex matrices because our own tongues operate with pores that open and close in response to chemical stimulation.[3] Even covalent capture strategies exist as in our amplifier approach to make the experience of hot spices an intense and long-lasting one.[4] Realized examples in the context of synthetic pores as artificial tongues include sucrose (glucose) sensors,[5] lactose sensors, lactate sensors, citrate sensors, acetate sensors, and glutamate (umami) sensors.[2] The applicability of the fluorometric detection of enzyme activity[6,7] toward inhibitor screening for drug discovery has been exemplified with hyaluronidase.[8]

Inositol phosphates (IP_n) and their enzymes were an exceptionally attractive family of analytes for multicomponent sensing with pores. Without an intrinsic chromophore, IP_n sensing and screening in a non-invasive naked-eye multiwell format is not easily achieved, yet desirable considering their diverse roles in biology and beyond. For example, 75% of the phosphate in corn seeds is stored in the form of phytate (inositol hexaphosphate, IP_6, Fig.).[9] The phosphates needed as essential biological building blocks are lost to poultry, swine, cattle, and humans who all lack the phytase enzymes which liberate phosphates, causing most of the IP_6 antinutrient to be excreted together with essential elements. The inorganic phosphate additives needed to compensate for this loss constitute today's phosphate crisis, which relates to the inefficient use of depleting and non-renewable phosphorous resources, in addition to water pollution. New sensors will be helpful to support efforts on phytate profiling and phytase bioengineering to address these problems. Other attractive sensing applications include the recently introduced IP_7, where one phosphate group in phytate is replaced by a pyrophosphate. IP_7 has proposed roles in bioenergetics (*i.e.* IP_7/IP_6 interconversion catalyzed by IP_6 kinase is a proposed ATP/ADP equivalent), in protein diphosphorylation, and in cellular signaling. However, progress in IP_7 research is hindered by analytical problems.[10]

Synthetic multifunctional pore **1** was selected to elaborate on IP_n sensing.[11] This pore is an artificial β-barrel, composed of rigid-rod staves that are held together by short β-sheets.[12] IP_n binding at the lysine- and histidine-rich inner barrel surface was found to block pore **1**. Blockage efficiency, described as IC_{50}, the inhibitory concentration needed to reduce pore activity to 50%, increased with increasing charge of the IP_n until phytate. The IC_{50} for phytate was 45 nM, sufficient for differential sensing in complex matrices without further amplification. To determine the phytate content in almonds, for example, the ability of extracts to block pore **1** was measured before and after treatment with phytase. Phytase was found to reduce the blockage efficiency of the extracts. The difference in pore blockage, before and after phytase treatment, results from blockage by phytase substrates (Fig.).

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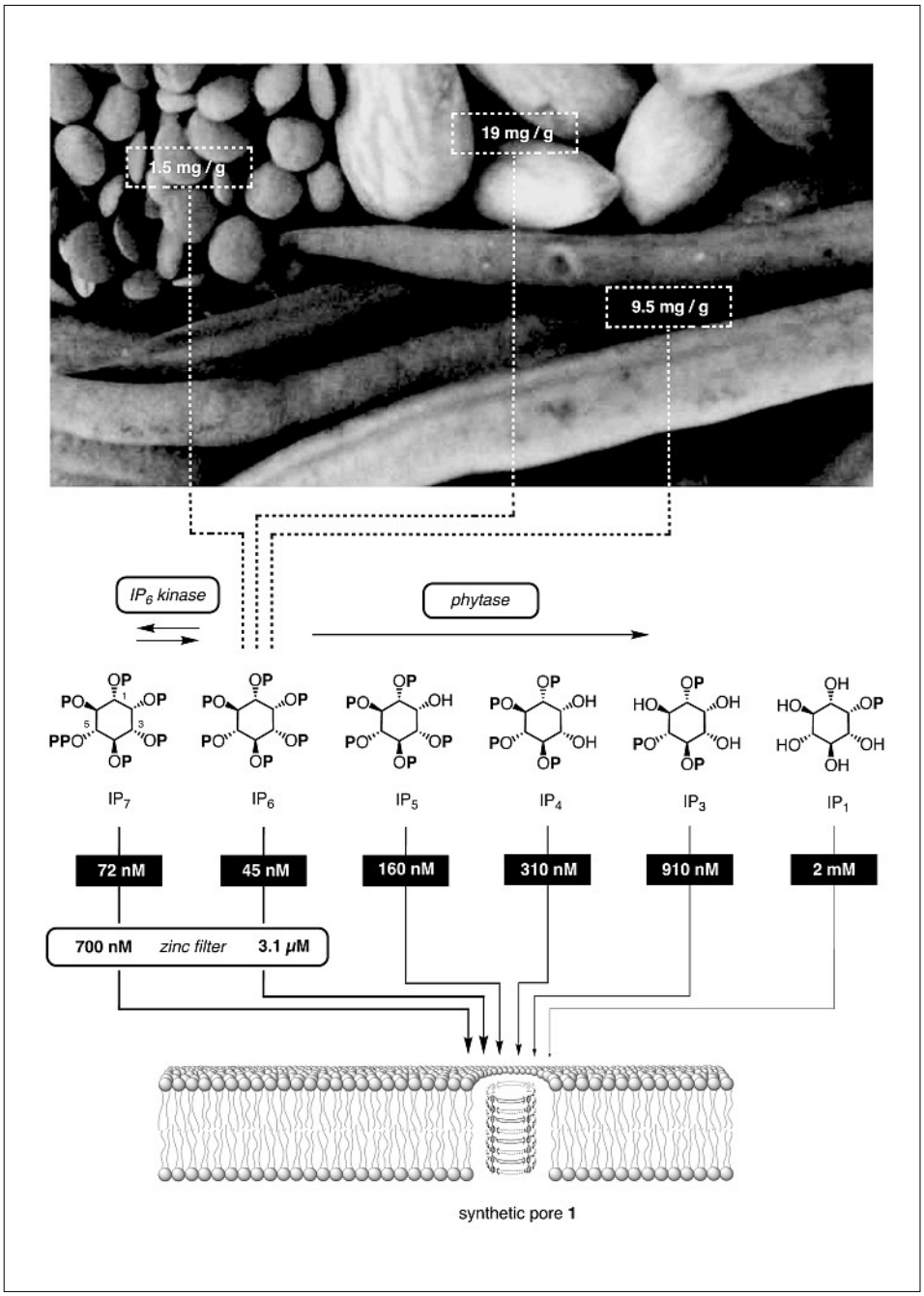

Fig. Synthetic pores as multicomponent sensors in complex matrices exemplified with differential phytate sensing using phytase as specific signal generator, and the discrimination of IP_7 and IP_6 using Zn^{2+} as a filter. The indicated concentrations are IC_{50}s (*i.e.* the IP_n concentration needed to reduce the activity of pore **1** to 50%) in the absence (white on black) and the presence (black on white) of ~30 μM $ZnCl_2$. **P** = $PO_3H_n^{-(2-n)}$, **PP** = $P_2O_6H_n^{-(3-n)}$, the indicated stereoisomers are the ones used for pore blockage and do not necessarily correspond to phytase products. The activity of the indicated enzymes is detectable with pore **1**; the phytate content determined in lentils, almonds, and soybeans is indicated.

With possible contributions from IP_ns other than IP_6, this value remains somewhat qualitative. However, the excess phytate present in plant samples, together with its superior blockage efficiency suggested that interference from IP_5, IP_4, *etc.*, on fluorometric phytase profiling in complex matrices is nearly negligible. The values obtained for almonds, lentils, and soybeans were excellent, only slightly below expectations from the literature and controls with less selective assays. Preliminary results supported the view that kinetic enzymatic discrimination or enzymatic cascade generators may already be sufficient to further dissect the different contributions from individual IP_ns, including IP_7, if desired.

IP_7, however, was another story. Whereas the discrimination of ATP and ADP, of paramount importance for the detection of kinase activity and beyond, was already a challenge to achieve with synthetic pores,[2,5,8] the discrimination of IP_7 and IP_6 appeared even more demanding. The IC_{50} values of the two were nearly the same. Their high efficiency suggested that this lacking discrimination might be obscured by stoichiometric binding,[13] and their high charge and fractional charge difference might be too small. Both problems, we expected, could be overcome with the addition of zinc chloride. Zn^{2+} binding to the IP_ns should reduce their charge and increase their IC_{50} values to make eventual discrimination possible. This turned out to be the case. The addition of Zn^{2+} filters increased the IC_{50} of IP_6 much more than that of IP_7. The obtained discrimination was clearly sufficient for the fluorometric detection of the activity of IP_6 kinase that catalyzes the IP_6 and IP_7 interconversion.

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